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RECENT DEVELOPMENTS IN STUDIES ON BIOLOGICAL FUNCTIONS OF VITAMIN A IN NORMAL AND TRANSFORMED TISSUES

Luigi M. De Luca, Sergio Adamo, Pangala V. Bhat, Wlodzimierz Sasak, Carol S. Silverman-Jones, Irene Akalovsky, Jacques P. Frot-Coutaz, Theodore R. Fletcher* and Gerald J. Chader*

National Cancer Institute and National Eye Institute*, Bethesda, MD, 20014

Abstract - A biochemical pathway of phosphorylation and glycosylation of vitamin A has recently been found in hepatic, intestinal and epidermal tissues. More recent work suggests that mannosylretinylphosphate functions as a donor of mannose to membrane glycoconjugates. These reactions might ultimately explain the effects of vitamin A deficiency and some of the effects of excess vitamin A on biological systems.

Studies of the effect of retinoids on cellular in vitro systems showed an increase in the adhesive properties of spontaneously-transformed mouse fibroblasts in culture (Balb/c 3T12-3 cells). These cells are usually detached from the culture dish surface in an EDTA adhesion assay. After culturing in presence of 3.3×10^{-6} to 3.3×10^{-5}M retinol or retinoic acid the cells are no longer lifted from the plate and their morphology and adhesion resemble those of normal fibroblasts. This phenomenon of increased adhesion is observed as early as two days after exposure to the retinoid and it is readily reversible upon culturing in medium without exogenous retinoid. A variety of retinoids was tested in the adhesion assay. The most active compounds were retinol, retinylphosphate, retinoic acid, 5,6-epoxyretinoic acid and the TMMP and DACP derivatives of retinoic acid. All these compounds possess biological activity in other systems. Anhydroretinol, perhydromonoeneretinol, the phenyl derivative of retinoic acid, which do not have biological activity in other systems, did not increase adhesion of 3T12 cells. Other polyprenoid compounds without vitamin A activity were also tested in this assay. Dolichol, dolichylphosphate juvenile hormone, abscisic acid, β-ionone, dibutyryl cyclic adenosine monophosphate and sodium butyrate did not induce adhesion.

The mechanism by which retinol and retinoic acid increase the adhesive properties of 3T12 cells was investigated. Cyclic adenosine monophosphate and guanosine monophosphate levels were not significantly altered by retinoid treatment at least at 6, 24, 48 and 72 hours after treatment with 3.3×10^{-5}M retinoic acid, when most of the cells remain attached. Retinoic acid stimulated the incorporation of (2-^{3}H) mannose into glycoproteins of 3T12 cells. (11, 12-^{3}H and carboxyl-^{14}C)Retinoic acid was incorporated into a compound (Metabolite I) which had chromatographic properties of a glycosylretinylphosphate. The synthesis of this compound was time-dependent and was not carried out by formalin -fixed 3T12 cells. Mild alkaline conditions which release anhydroretinol from retinylphosphate, also cleaved Metabolite I to yield a product with the polarity of a hydrocarbon, but slightly more polar than anhydroretinol. It is suggested that retinoic acid can be reduced to an alcohol, probably after metabolic modification. It is further suggested that such "retinol-like" compound would follow the same route of phosphorylation and glycosylation as shown for retinol in other systems. Microsomes from 3T12 cells were active as the intact cells in synthesizing mannosylretinylphosphate and dolichyl mannosylphosphate. Exogenous retinylphosphate specifically stimulated the synthesis of mannosylretinylphosphate.

Thus it appears that vitamin A is involved in glycosyl transfer reactions in the 3T12 system, as well as in normal membranes. It remains to be established whether the observed increased adhesion is the result of such involvement.

A novel reaction for retinol was found in 3T12 cells. Up to 55% of exogenously supplied retinol was converted to the hydrocarbon anhydroretinol in 48 hours. The same reaction was also carried out by microsomes from 3T12 cells, which converted 7% of retinol to anhydroretinol in 30 minutes at 37°C. This reaction may well represent a detoxification mechanism for the transformed cell.

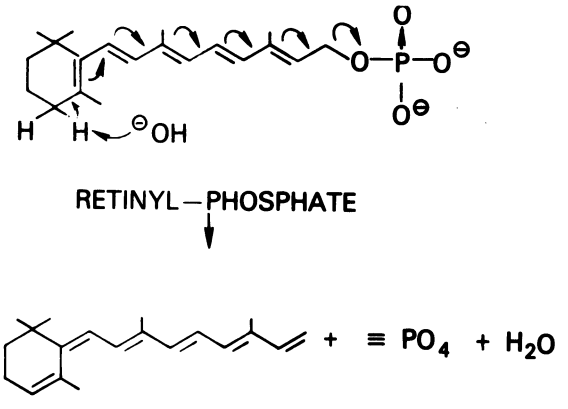


Fig. 2. Proposed reaction mechanism for the alkaline hydrolysis of retinyl phosphate to anhydroretinol.

chromatography on columns of DEAE-cellulose acetate. Mannosylretinylphosphate is eluted with 10mM ammonium acetate, whereas retinylphosphate is eluted at 30mM salt.

Mannosylretinylphosphate

This compound is synthesized in vivo in rat and hamster liver and in vitro by cultured epithelial and mesenchymal cells (9). It is separated from dolichylmannosyl-phosphate (DMP) on thin layers of silica gel in chloroform/methanol/water (60/35/6), in which it has Rf 0.3-0.4, whereas DMP has Rf 0.6-0.7. Remarkably, MRP is extracted selectively from intact cells with 99% methanol, a solvent which leaves DMP unextracted.

In addition to epithelial cells, fibroblasts are also very active in synthesizing MRP. Exogenous retinylphosphate specifically stimulates MRP synthesis. This compound is also very labile, and contact with oxygen should be avoided. Storage is most effective in methanol at 4°C under nitrogen.Recent work has shown that administration of retinol or retinoic acid to vitamin A depleted rats increases the in vivo synthesis of MRP by 2 to 3 fold within one hour. This response is as fast as the secretion of RBP from the liver of these same rats (3,21). However, retinoic acid, a compound which does not induce secretion of hepatic RBP into the blood stream, also enhances the biosynthesis of MRP. Whether a reduced metabolite of retinoic acid (X) is involved in the synthesis of MXP, is an interesting possibility, which has received experimental support as discussed later (fig. 5).

Synthetic retinoylphosphate

The question was asked as to whether or not phosphorylated retinoic acid could function as acceptor of mannose to replace retinylphosphate. Synthetic retinoylphosphate and its 13-cis isomer did not function in vitro as mannosyl acceptors (9). This finding supports the idea that retinoic acid may play a role in glycoprotein synthesis only after conversion to a reduced "retinol-like" metabolite X (fig. 5).

Other glycosylretinylphosphates.

A search for the synthesis of other glycosyl retinylphosphates (22) has led to the conclusion that mannosylretinylphosphate is the only compound synthesized in vitro by rat liver microsomes. UDP-galatose, UDP-glucose, CMP-sialic acid, UDP-N-Acetyl-glucosamine and UDP-N-Acetyl-galactosamine were not active in donating their glycosyl residues to exogenous retinylphosphate. It is possible, however, that a metabolically modified form of the vitamin may act as a carrier of these other monosaccharides as well. It should also be considered that retinylphosphate may carry different monosaccharides in different tissues. Thus a galactosylretinylphosphate from mastocytoma cells has been reported (23). Moreover retinylphosphate enhances the incorporation of galactose from UDP-galactose into rhodopsin (24).

Mannosylretinylphosphate as a donor of mannose.

The synthesis of retinylphosphate has greatly aided the investigation of the reaction of transfer of mannose to endogenous membrane acceptors. This work is still in progress, but considerable advances have been made in the recent past (25,26). It has been established that MRP transfers mannose to membrane glycoconjugates which are sensitive to the action of proteases. These endogenous acceptors represent a family of products ranging in molecular weight from over 90,000 to 20,000 daltons, at least in rat liver membranes. [^{14}C] Mannosylretinylphosphate does not function as a donor of mannosyl residues after irradiation with UV light or in the presence of an inactivated boiled enzyme. The bond formed between [^{14}C]mannose and the acceptor is stable to 0.1N NaOH at 37°C for 20 minutes and to 0.01 N HCl at 90°C for 30 minutes, conditions which do not cleave most glycosidic bonds. A major advance in the elucidation of the nature of

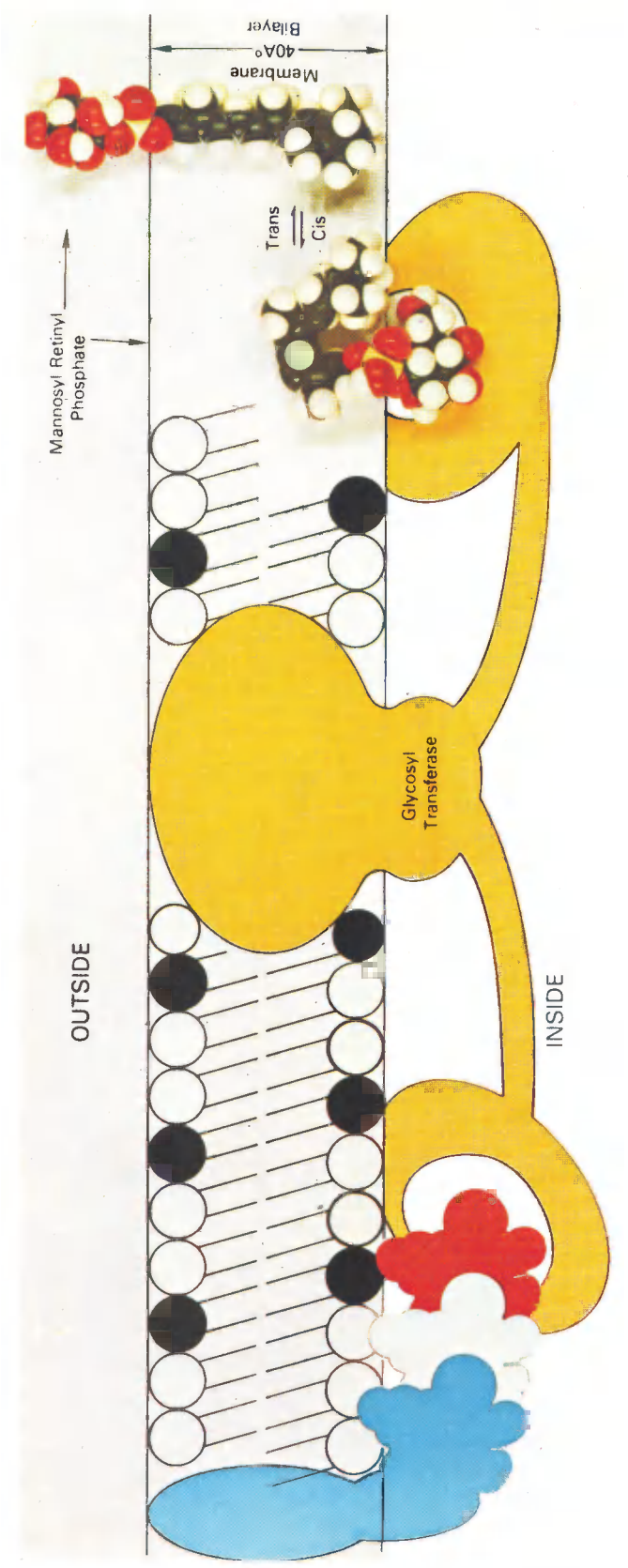


Fig. 1. WORKING SCHEME FOR A TRANS-CIS MEMBRANE CROSSING ACTION OF VITAMIN A IN GLYCOSYL TRANSFER REACTIONS.

INTRODUCTION

Vitamin A (retinol) is an essential nutrient present in the diet either as such or in the form of its precursor molecule, β-carotene. This molecule is cleaved by an intestinal dioxygenase to yield vitamin A aldehyde (retinal), which is then reduced in the mucosa to the alcohol retinol. Vitamin A is transported to the liver, where it is stored as retinyl palmitate (1,2). When called upon because of tissue needs, the ester is hydrolyzed, and retinol is secreted into the blood in complex with a specific binding protein (RBP) of approximate molecular weight 21,000 daltons (3). This protein associates with prealbumin to form a complex of molecular weight 80,000 daltons which prevents the loss of RBP through the glomerular membrane of the kidney (4). It is the function of RBP to deliver vitamin A to target tissues such as the pigment epithelium (5) of the eye. Specific cellular binding proteins for retinol (6) and retinoic acid (7) appear to play a role in the transfer of the vitamin from the blood into the tissue. Investigations into the systemic mode of action of vitamin A, outside vision (8), have only recently provided some promising leads which may explain some of the biological functions of vitamin A. In particular, retinylphosphate has been shown to be involved in glycosyl transfer reactions of mammalian membranes (9). Recent advances in this field will be presented here.

Retinoids have also been shown to possess chemopreventive activity in models of chemical carcinogenesis (10) and to alter growth characteristics of neoplastic cells in culture (11, 12, 13). Retinoid-treated cells appear to gain growth properties of "normal" cells with a lower saturation density and an increased adhesion (13) to each other and to the tissue culture dish. The adhesion assay in the 3T12 cell system will be presented as a new assay for the biological activity of retinoids. Studies on the metabolism of retinol and retinoic acid and a correlation between the increased adhesion and the stimulation of the biosynthesis of mannose-containing glycoproteins of 3T12 cells will also be presented.

INVOLVEMENT OF VITAMIN A IN GLYCOPROTEIN SYNTHESIS

Studies described elsewhere (9) have led us to conclude that vitamin A is directly involved in the biosynthesis of specific glycoproteins. The main goblet cell glycoprotein "gobletin" has been identified as vitamin A dependent in the course of these studies (9,14). The incorporation of radioactive mannose into glycoproteins of liver tissue is severely (60 to 90%) decreased in vitamin A deficient animals (15) and enhanced (200 to 300%) in animals receiving high doses of retinyl palmitate (16). Both retinol and retinoic acid stimulate mannose incorporation into glycoproteins (9). Thus, it should be considered that they act in glycoprotein biosynthesis either through a common intermediate or by two parallel pathways. We proposed (17) that retinylphosphate may act as a carrier of monosaccharides across the hydrophobic environment of the membrane bilayer (fig.1). The phosphate moiety would stick out of the hydrophobic environment into the hydrophilic environment and be amenable to glycosylation. Glycosylretinylphosphate would then isomerize to a cis configuration (e.g.9-cis) and allow crossing of the membrane. On the membrane side ("Inside" of Fig. 1) the sugar moiety would then be recognized by the glycosyl transferase and transferred to the growing polysaccharide chain of glycoproteins (fig. 1). This working hypothesis was strengthened by the demonstration that retinyl phosphate, the postulated carrier, is synthesized in vivo and by cellular systems in vitro (9, 18).

Retinylphosphate

The most extensively studied tissues for the biosynthesis of retinyl phosphate have been the intestine, the liver and the epidermis (9). These studies have established that vitamin A is phosphorylated in vivo and in cultured cell systems, without prior oxidation of the carbinol function. In a study with a pooled sample from 30 mucosal linings from hamster intestine, retinylphosphate was measured spectrophotometrically giving a concentration of about 35 ng/gram of wet mucosal lining. Its activity in reversing squamous metaplasia and keratinization of vitamin A depleted hamster tracheal organ cultures was also demonstrated (18). Chemical retinylphosphate is active in promoting growth of vitamin A depleted rats (19). However, recent studies from our laboratory show that the half life of retinyl phosphate in cultures of 3T12 cells is about 30 minutes, thus any measurement of biological activity of this compound in intact cell systems is probably a function of the activity of the retinol released by hydrolysis of retinyl phosphate. Both the ultraviolet absorption spectrum and the fluorescence emission and excitation spectra of retinylphosphate are identical to those of retinol.
The finding that retinylphosphate is readily cleaved by 0.1 N NaOH at 37°C for 20 minutes to yield anhydroretinol (fig.2), a compound with six conjugated double bonds, has aided in the characterization of small amounts of biosynthetic retinyl phosphate. The easiest and most reliable isolation procedure is summarized elsewhere (20).

Even using this relatively fast procedure the breakdown of retinylphosphate during isolation is more than 50% of the starting material.
Retinylphosphate is separated from its glycosylated derivatives by anion change

the glycoprotein acceptors was made, when it was found (27) that 80% of the acceptors were cleaved under alkaline conditions in the presence of $NaBH_4$ (0.05 M NaOH, IM $NaBH_4$ for 12 hours at 45°C). These conditions are considered optimal for cleavage of the serine and threonine O-glycosidically-linked oligosaccharide chains. About 20% of the [^{14}C] labeled macromolecular acceptors are resistant to this alkaline cleavage and may belong to the family of glycoproteins which contain the N-glycosidic glycopeptide bond. It should be pointed out that dolichylmannosylphosphate, the polyprenoid (C_{85}-C_{100}) lipid intermediate has been shown to generate mannosyl residues in glycoproteins which contain the asparaginyl-linked N-glycosidic glycopeptide bond, not amenable to alkaline cleavage (28). In a double label experiment using [^{14}C] mannosylretinylphosphate and dolichyl [^{3}H] mannosylphosphate as donors, it was shown that alkaline treatment of the macromolecular acceptors for mannose released different populations of products from the two lipid intermediates.

This involvement of vitamin A in glycoprotein synthesis may well explain most of the biological effects of the vitamin. It also appears to mediate the increased adhesion of transformed cells to substratum, as will be discussed.

THE NEOPLASTICALLY TRANSFORMED MOUSE FIBROBLAST SYSTEM (BALB/c 3T12-3 CELLS)

Recently, work from several laboratories has demonstrated that retinoids affect the growth properties of cultured transformed cells (11,12,13). In general, transformed cells tend to grow without contact inhibition. Thus, spontaneously-transformed mouse fibroblasts (Balb/c 3T12-3 cells) grow to a density of about 450,000 cells /cm^2. However, when cultured in the presence of retinoic acid, they reach a lower saturation density, while their plating efficiency remains the same. Moreover the morphology of the retinoid-treated cells more closely resembles that of normal fibroblasts in culture. They appear flat by transmission electron microscopy (fig. 3B), compared to their untreated counterparts (fig. 3A).

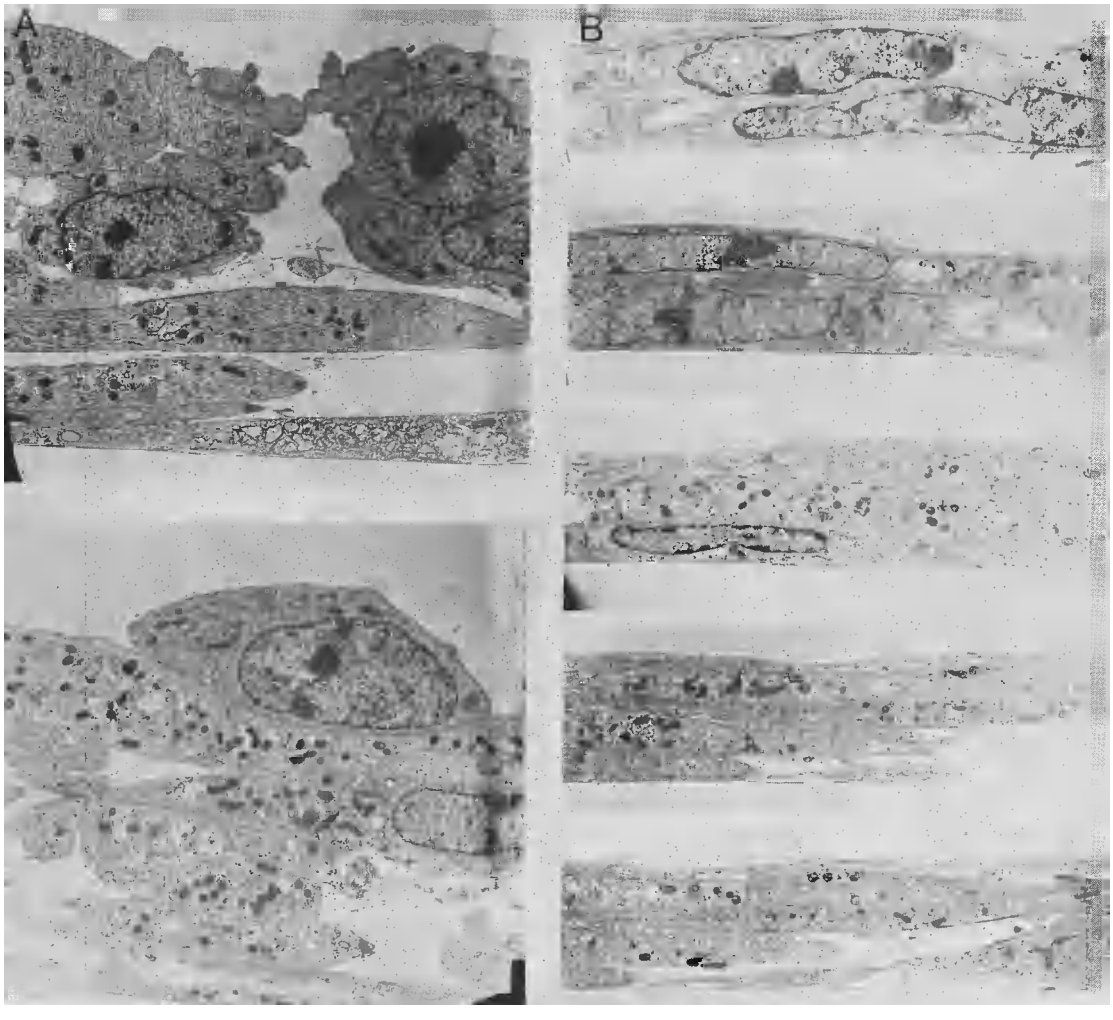


Fig. 3 Transmission electron microscopy of control (A) and retinoic acid treated (B) 3T12 cells. Cells were grown for seven days.(x3,500).

This observation was made more interesting by the finding that the adhesive properties of retinoid treated cells were increased dramatically (29). A quantitative EDTA assay to measure adhesion was developed by Adamo et al (29). Between 90 and 100% of the untreated cells are detached by treatment with EDTA, whereas retinoid treatment at 3.3×10^{-5}M causes 90% or more of the cells to remain attached to the plate. Dose-response curves for this effect of retinol and retinoic acid are shown in figure 4.

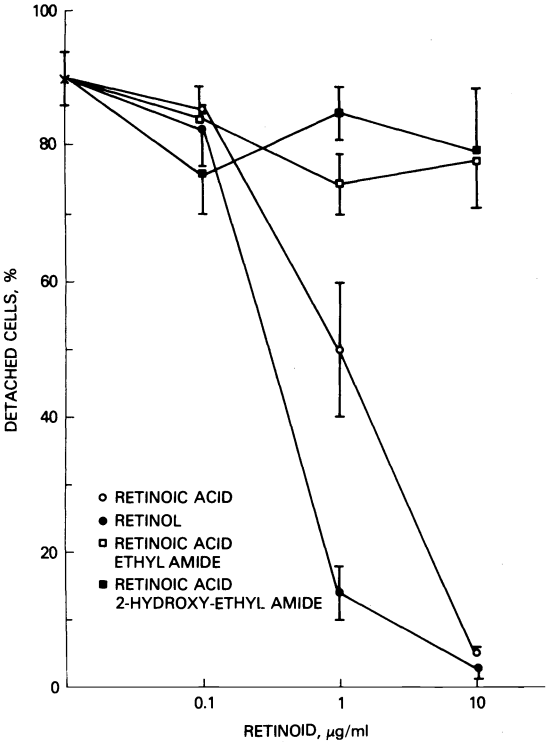


Fig. 4 Dose response curves for retinol and retinoic acid induced adhesion of 3T12 cells. The ethylamide and the 2-hydroxy-ethylamide were inactive.

In this assay cells are incubated with the retinoid for 6 days to obtain maximal effect, although the effect is detectable two days after the addition of the retinoid (29). All compounds with vitamin A activity are active in the adhesion assay. Table I rankorders the various compounds tested: retinylphosphate and retinol are the most active compounds; retinoic acid, 13-cis retinoic acid, the 5,6-epoxy retinoic acid, the DACP and TMMP derivatives of retinoic acid (Table I) were all active. These compounds have been shown to be active in preventing squamous metaplasia induced by vitamin A deficiency in the tracheal organ culture system (30). The phenyl derivative, inactive in the tracheal system, is also inactive in inducing adhesion. Equally inactive in the adhesion system were esters and amides of retinoic acid, probably because of the lack of secreted hydrolases in these cells: figure 4 shows the results obtained with the ethyl amide and the 2-hydroxyethylamide of retinoic acid. Other polyprenoid derivatives without vitamin A biological activity were tested in the adhesion assay. The polyprenol dolichol and its phosphate derivative were both inactive. Juvenile hormone (not included in Table I) and abscisic acid were also inactive.

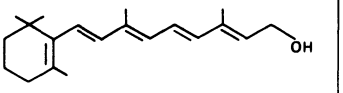
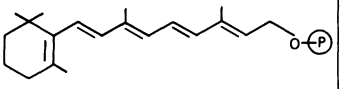
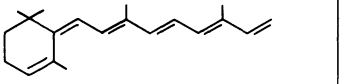
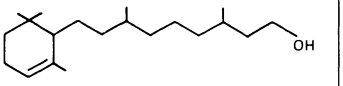
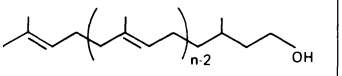
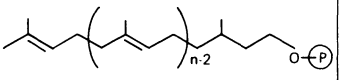
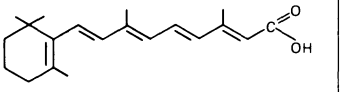
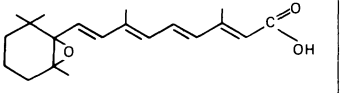
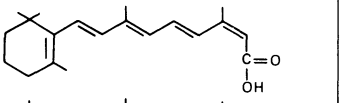
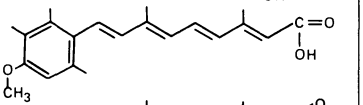
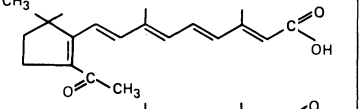
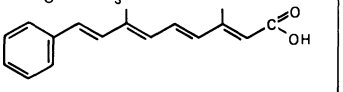
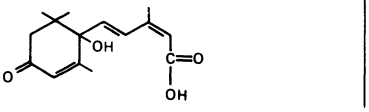
Retinoic acid and its derivatives were a kind gift from Dr. Beverly Pawson of Hoffmann-La Roche Inc., Nutley, NJ

In conclusion the fibroblast adhesion assay is a new assay to measure the biological activity of retinoids and their metabolites. It also is a simple in vitro system to investigate the biochemistry of vitamin A.

Cyclic-adenosinemonophosphate and guanosinemonophosphate in 3T12 cells.

It was of interest to measure c-AMP and GMP levels, since Johnson and Pastan (31) had reported that c-AMP increases the adhesion of fibroblasts to substratum. Cyclic AMP and GMP were measured in retinoic acid treated (3.3×10^{-5}M) and untreated 3T12 cells at 6,24,48 and 72 hours after the administration of retinoic acid. Retinoic acid was added 24 hours after seeding the cells. The c-AMP and c-GMP were measured in triplicate by the method described by Fletcher and Chader (32). No significant difference in the levels of c-AMP or c-GMP was detected.

BIOLOGICAL ACTIVITY OF RETINOIDS IN THE ADHESION ASSAY OF SPONTANEOUSLY-TRANSFORMED MOUSE FIBROBLASTS IN CULTURE (Balb/c 3T12-3 cells).

Name	Structure	Activity At 0.1 μg/ml	Activity At 1.0 μg/ml	Activity At 10.0 μg/ml
Retinol		–	++	++
Retinyl Phosphate		–	++	++
Anhydroretinol		–	–	–
Perhydromonoene Retinol		–	–	–
Dolichol (n = 16-20)		–	–	–
Dolichyl Phosphate		–	–	–
Retinoic Acid		–	+	++
5-6 epoxy Retinoic Acid		–	+	++
13-cis-Retinoic Acid		–	–	++
TMMP-Retinoic Acid		–	–	++
DACP-Retinoic Acid		–	–	++
Phenyl Analog of Retinoic Acid		–	–	–
Abscisic Acid		–	–	–

– No Effect (70 to 100% of cells detached)
\+ Moderate Effect (30 to 70% of cells detached)
++ Large Effect (0 to 30% of cells detached)

Table 1.

Table 2. Cyclic nucleotide levels (pMoles/mg protein ±SEM) in spontaneously-transformed mouse fibroblasts. Assays were in triplicate.

	No Treatment ± S.E.M.	DMSO ± S.E.M.	Retinoic Acid ± S.E.M.	Time After Retinoic Acid
c-AMP	1.5 .3	1.4 .3	1.1 .2	6 hrs.
c-GMP	0.09 .02	0.09 .02	0.10 .02	
c-AMP	2.3 .6	2.4 .5	1.3 .1	24 hrs.
c-GMP	0.10 .01	0.11 .01	0.1 .01	
c-AMP	1.3 .2	1.5 .1	1.2 .1	48 hrs
c-GMP	0.10 .01	0.08 .01	0.09 .01	
c-AMP	3.4 .2	2.0 .3	1.9 .2	72 hrs
c-GMP	0.08 .01	0.11 .01	0.14 .01	

The metabolism of retinol in transformed mouse fibroblasts.

A new enzymatic reaction, the dehydration of retinol to anhydroretinol was found in 3T12 cells (33). Up to 55 percent retinol is converted to this hydrocarbon within 48 hours of culture. The compound was identified by gasliquid chromatography and UV spectrometry which showed its typical absorption triplet at 386,364 and 346 nm. A microsomal preparation from 3T12 cells also converts retinol into anhydroretinol (33) Boiled microsomes or formaline fixed cells do not carry out this dehydration reaction. The reaction also occurs in cultured 3T3 cells and in primary mouse dermal fibroblasts, but to a much lesser extent. Anhydroretinol appears to be an end product of the metabolism of retinol since it is recovered intact at the end of a 30 hour incubation with 3T12 cells, without conversion to retinol or retroretinol.
In contrast to these fibroblastic systems, cultured intestinal epithelial cells from the hamster do not synthesize anhydroretinol from retinol. The major metabolic product of these cultured cells is a retinyl ester .

Hydrolysis of retinylphosphate by 3T12-3 cells

Since retinylphosphate is active in increasing the adhesion of 3T12 cells, it was of interest to study its metabolism in this system. [^{3}H] Retinylphosphate was converted into [^{3}H] retinol (about 80%) and anhydroretinol (about 10%) within one hour of incubation with intact cells. Formaline-fixed cells did not carry out the reactions.

Biosynthesis of mannosylretinylphosphate and mannoproteins in 3T12-3 cells and their microsomes.

Intact transformed mouse fibroblasts synthesize mannosylretinylphosphate (MRP) and dolichylmannosyl phosphate (DMP). Microsomal preparations from these cells retained these biosynthetic activities. Addition of exogenous retinylphosphate stimulated the incorporation of [^{14}C] mannose from its sugar nucleotide precursor guanosinediphosphate [^{14}C] mannose, without increasing the incorporation of this sugar into the dolichylmannosylphosphate, as described for other systems (9).
Using [2-^{3}H] mannose as a precursor, it was shown that retinoic acid stimulates the incorporation of this sugar into glycoproteins of 3T12 cells by 1.6 fold.

The mode of action of retinoic acid.

Since retinoic acid is active in inducing adhesion of 3T12 cells, and in stimulating [2-^{3}H] mannose incorporation into glycoproteins (15,21 and previous paragraph) it was of interest to investigate the possibility that the acid also participates in the glycosyl transfer pathway. Figure 5 proposes that retinoic acid is first modified (e.g. by epoxidation, 34), and then reduced to a retinol-like derivative (X). Metabolite X contains a carbinolic function and can undergo the same series of phosphorylation and glycosylation reactions as retinol itself. Alternatively, the idea that retinol could be

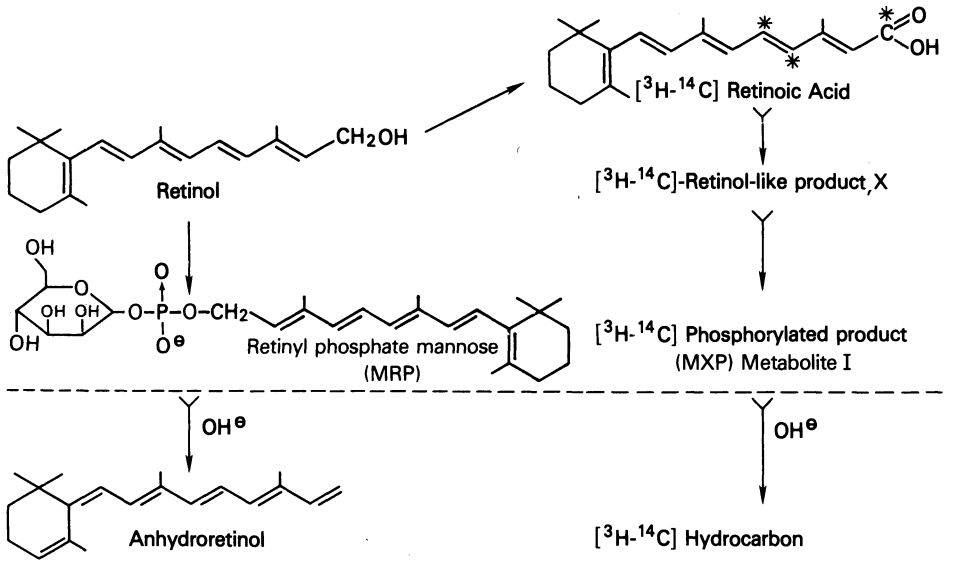


Fig. 5. Proposed comparative scheme for the metabolism of retinoic acid and retinol.

converted to Metabolite X via retinoic acid is less likely in view of the finding that [^{3}H-carbinol]retinol is converted to [^{3}H-carbinol] retinyl phosphate in hamster intestinal cells (18) and in mouse epidermal cells (20) without loss of tritium, i.e. without oxidation to retinoic acid. Doubly labeled [11,12-^{3}H and carboxyl-^{14}C] retinoic acid was used for some of these studies. This compound was taken up by the growing fibroblasts. Approximately 6.1% of the retinoid was taken up in 96 hours. Formaline fixed cells did not incorporate the retinoid. About 90% of the incorporated retinoid was in the form of intact retinoic acid; 4% had been converted into a metabolite (Metabolite I) which had the same chromatographic properties as mannosylretinylphosphate. Since the hydrolysis of retinylphosphate and its glycosidic derivatives yields anhydroretinol (fig. 2), if Metabolite I contains a "retinol-like" compound (X of fig.5), it might yield the hydrocarbon of X under the same conditions. The product of mild alkaline hydrolysis of Metabolite I had the polarity of a hydrocarbon upon chromatography on alumina (Brockman Grade III), but could be separated from anhydroretinol by chromatography on thin layers of silica gel in hexane/acetone (90/10) in which anhydroretinol had Rf0.64 and the hydrolysis product of Metabolite I had Rf0.49. Moreover, double label studies using (11,12-^{3}H) retinoic acid and [^{14}C] mannose have allowed the isolation of a double labeled compound with chromatographic and hydrolytic properties of standard MRP. Inasmuch as this compound contains Metabolite X, it should be referred to as MXP. Thus MXP and Metabolite I are the same product. These studies represent the first demonstration of a molecular involvement of retinoic acid in the biosynthesis of a mannolipid.

CONCLUSIONS

A variety of biological alterations occur as a consequence of vitamin A deficiency. These could be due to the same or different biochemical mechanisms. It appears that a likely explanation for many biological effects of vitamin A may reside in its recently discovered function in the biosynthesis of glycoproteins, since both retinol and retinoic acid are active in maintaining glycoprotein biosynthesis in a variety of tissues (9).

The recent finding that retinoids affect the adhesion of spontaneously-transformed mouse fibroblasts has permitted the definition of a new assay system for their biological activity. Interestingly, retinylphosphate and retinol are the most active compounds. Retinoic acid and its DACP, TMMP and 5,6 epoxyderivatives, as well as its 13-cis isomer are also active (Table 1). Amide and ester derivatives of retinoic acid were inactive in this system. Compounds without growth-promoting activity such as anhydroretinol, perhydromonoene-retinol and the phenyl derivative of retinoic acid were not active. Inactive were the polyprenoids dolichol, dolichyl phosphate, and abscisic acid, juvenile hormone, β-ionone, cyclic-AMP dibutyrate and sodium butyrate.

The biochemical mechanism by which retinol and retinoic acid act in this vitro system was also investigated. The levels of cyclic-adenosinemonophosphate and guanosinemonophosphate were the same in adhesive and non-adhesive cells (Table II), suggesting that retinoid-induced adhesion does not involve these compounds.

Instead, retinol and retinoic acid were found to increase (2-^{3}H) mannose incorporation into glycoproteins of 3T12 cells. A compound with chromatographic characteristics of mannosylretinylphosphate was isolated from the methanolic extract of these cells. Its synthesis in a 3T12 microsomal system was stimulated by exogenous retinylphosphate. Retinoic acid was also incorporated into a compound with the same chromatographic characteristics as MRP in the intact cell system (MXP or Metabolite I of fig. 5). Hydrolysis data suggest that retinoic acid is reduced to a "retinol-like" compound (X of fig. 5) and that X undergoes the same reactions of phosphorylation and glycosylation as retinol itself to yield MXP.

Finally, in the course of these studies a novel enzymatic reaction of retinol was found: its dehydration to anhydroretinol. This reaction was not found to occur in cultured intestinal cells, which converted retinol mainly into retinyl esters.
It is concluded that, in addition to its action on epithelial systems, vitamin A may also control the growth and adhesion of some mesenchymal cells. It is suggested that these different biological functions of vitamin A may well result from the involvement of the vitamin in glycosyl transfer reactions.

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